CXXXII. COMPARISON OF THE EFFECTS OF ARGININE AND THYROXINE UPON TUMOUR GROWTH-RATE IN THE MOUSE.

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THE growth rate of a tissue may be considered to depend in part upon the amount and nature of the substances available for its nutrition. If it is found that a specific chemical substance accelerates tissue growth it should be possible to reverse this effect provided that the concentration of such a substance in circulation could be reduced.

In the case of tumour tissue there is experimental evidence that arginine induces an increase in the rate of growth [Gilroy, 1930], and it is probable that this amino-acid is necessary for the nutrition of any tissue in which cell reproduction is proceeding. It has been shown [Crabtree, 1928] that the glycolytic activity of tumours is not a specific attribute of malignant tissue, other pathological overgrowths exhibiting the same type of carbohydrate metabolism in varying degree. Since normal tissues may temporarily assume the characters of neoplastic tissues in this respect it might be assumed that they would show coincident similarities in the metabolism of protein.

Assuming therefore that a certain level of arginine concentration is necessary for the adequate nutrition of tumour tissue, and that other tissues may under special circumstances demand the same material, for reproduction or repair, it was evident that one method of reducing the amount of arginine available for the tumour would be to divert it by creating such a demand.

It is known that administration of the active principle of the thyroid to healthy subjects causes an increased excretion of nitrogen, chiefly in the form of urea [Rohde and Stockholme, 1919] and that unless the protein content of the diet is high more nitrogen is excreted than ingested, indicating that tissue protein is being destroyed [Cushny, 1924]. In view of these facts it seemed reasonable to postulate an increased catabolism of protein under the influence of thyroxine, which might affect the tumour either directly, or by stimulating metabolism in general to an extent which brought the whole body into competition for such substances as were necessary for growth and tissue repair. Such an hypothesis would also accommodate an observation (Gilroy, unpublished) that the rate of tumour growth in the pregnant mouse is exceedingly slow; under such conditions embryonic tissue is present requiring nourishment

similar to that demanded by the tumour, and the metabolism of the body as a whole is slightly increased as a result of the pregnant condition.

Experiments were therefore undertaken to test the effect of non-toxic doses of thyroxine alone and in combination with arginine. The technique and material were similar to those used in a previous investigation [Gilroy, 1930]. The calipers were specially made for the purpose of obtaining accurate measurements, with a screw adjustment (40 threads to the inch)[1].

Exp. 1. 30 mice implanted 10 days previously were divided into 3 groups. Group I received 0·3 cc. of a 10 % solution of arginine every second day. Group II were given similar treatment, but in addition were injected daily with 0·1 cc. of a 1/1000 solution of thyroxine[2], the arginine being given in the morning and the thyroxine at night. Group III were untreated controls. Caliper measurements were made on the first and last days of the experiment, 10 and 22 days respectively after implantation, and cross-sectional surface area was determined in mm.^{2}; the mice were also weighed on the same dates.

This preliminary experiment gave the following result:

Group I. Arginine:

Mean size of tumours, day 10	... ...	14·6 mm.^{2}
,, ,, ,, 22	... ...	308·2 mm.^{2}
Mean weight of mice, ,, 10	... ...	21·6 g.
,, ,, ,, 22	... ...	24·5 g.

Group II. Arginine + thyroxine:

Mean size of tumours, day 10	... ...	23·5 mm.^{2}
,, ,, ,, 22	... ...	127·4 mm.^{2}
Mean weight of mice, ,, 10	... ...	20·9 g.
,, ,, ,, 22	... ...	24·1 g.

Group III. Untreated controls:

Mean size of tumours, day 10	... ...	13·6 mm.^{2}
,, ,, ,, 22	... ...	204·8 mm.^{2}
Mean weight of mice, ,, 10	... ...	22·5 g.
,, ,, ,, 22	... ...	25·5 g.

The ratio of size on the 10th and 22nd days respectively was:

Arginine	1 : 21·1
Arginine + thyroxine	1 : 5·3
Untreated controls	1 : 15·0

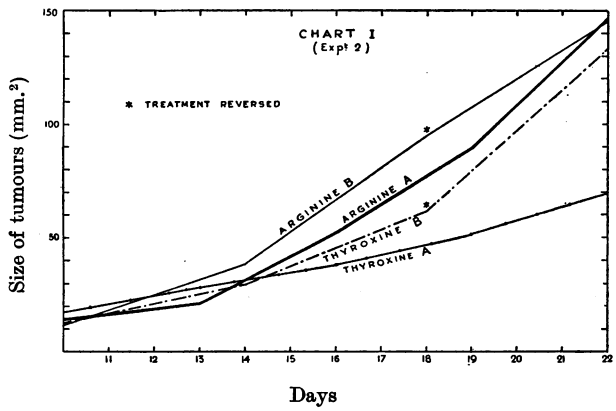
Taking the ratios as an index of the rate of growth it will be seen that arginine induced a more rapid rate of growth than occurred in the untreated control group, but that the latter again exceeded that of the group receiving thyroxine. In view of the small number of animals used in this initial experi-

[1] Made by Messrs A. W. Hall and Son, Engineers, Edinburgh.

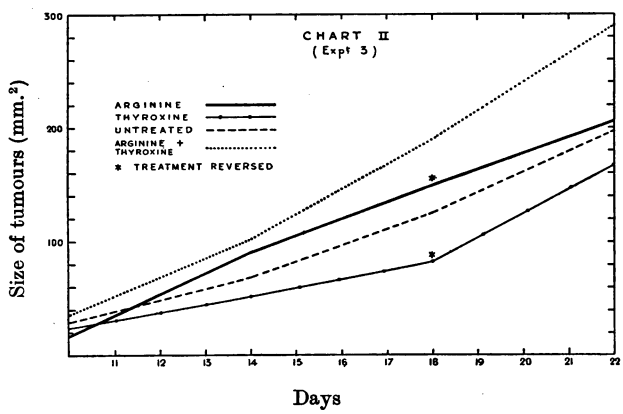
[2] Schering.

ment no reliance could be placed upon the results, but they were considered sufficiently encouraging to warrant further investigation upon a more adequate scale. In the succeeding experiments it was found that if arginine was given daily in conjunction with thyroxine the retarding effect of the latter was less marked (*e.g.* Exp. 4). The weights of the mice on the last day include the tumour, but it will be seen that in the arginine-treated group where the tumours were largest the increase in weight during the experimental period was no greater than in the group receiving thyroxine.

Exp. 2. 44 mice implanted 10 days previously were divided into two sections A and B, the sections being implanted from two different tumours. In each section one group was injected with arginine and the other with thyroxine but in section B treatment was reversed 18 days after implantation. The results are shown in Chart I.



Exp. 3. 46 mice implanted 10 days previously were divided into 4 groups, I receiving arginine, II thyroxine, III arginine a.m. and thyroxine p.m.; group IV were untreated controls. Treatment was reversed on the 18th day; the results are shown in Chart II.



Exp. 4. 45 mice implanted 10 days previously were divided into groups and treated as follows:

Group I. Arginine (A) 6 mice. Treatment reversed 18th day.
(B) 6 mice. Treatment continued 18th day.

Group II. Thyroxine (A) 6 mice. Treatment reversed 18th day.
(B) 6 mice. Treatment continued 18th day.

Group III. (A) arginine injected daily (6 mice).
(B) arginine injected every 2nd day (6 mice).

Group IV. Untreated controls (9 mice).

The result of this experiment is shown in Charts III, IV and V in which the first (*i.e.* treated) groups are shown separately for comparison with group IV (untreated controls).

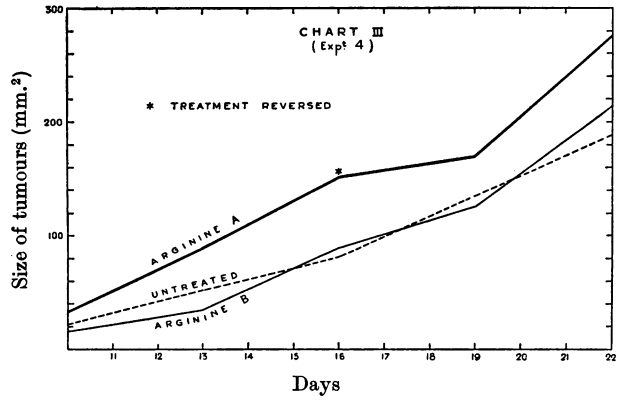
A summary of the protocols of Exps. 2, 3 and 4 is presented in the following table:

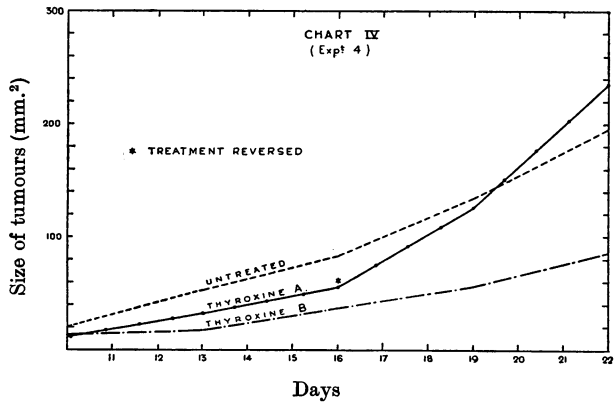
Summary of protocols.

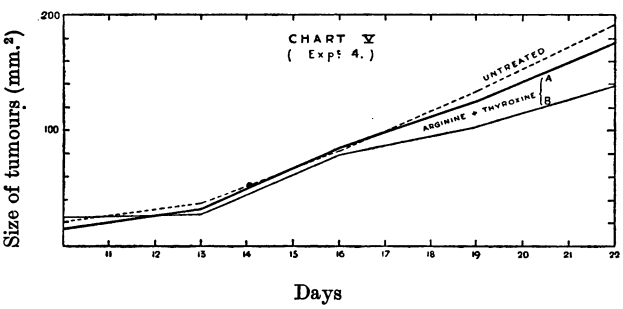
		Mean size of tumours		Mean weight of mice	
Exp.	Treatment received	Day 10 (mm.²)	Day 22 (mm.²)	Day 10 (g.)	Day 22 (g.)
II	Arginine (A)	14·5	146·5	24·3	28·3
	Thyroxine (A)	17·2	69·5	24·0	25·0
	*Arginine (B)	11·5	146·0	22·4	25·2
	*Thyroxine (B)	12·0	132·1	22·3	24·8
III	*Arginine	18·5	209·0	21·4	24·3
	Arginine + thyroxine	37·5	295·5	22·0	26·5
	*Thyroxine	26·8	169·8	22·6	25·1
	Untreated	30·7	202·3	20·4	22·8
IV	*Arginine (A)	33·8	276·6	22·1	23·6
	Arginine (B)	15·6	215·1	19·6	20·2
	*Thyroxine (A)	12·0	235·0	19·8	22·9
	Thyroxine (B)	12·5	85·5	19·0	20·4
	Arginine + thyroxine (A)	16·3	175·3	21·5	21·7
	Arginine + thyroxine (B)	26·8	139·4	20·8	22·0
	Untreated	20·7	193·4	19·7	21·7

* Treatment reversed during the 12-day experimental period.

In Exps. 2 and 3 reversal of treatment 18 days after implantation was followed by a deflection of the curve in the corresponding groups. As only one reading was possible after reversal it was doubtful whether the alteration would be maintained; in Exp. 4 an attempt was made to settle this point and the results show that the administration of arginine to a group previously receiving thyroxine caused a permanent increase in growth, and that if thyroxine was subsequently given to a group previously injected with arginine a temporary arrest of growth occurred but it was not maintained. If arginine was given daily in conjunction with thyroxine the rate of growth hardly differed from that of the controls; if given every second day the growth was correspondingly less.







Discussion.

The results of these experiments support the hypothesis that arginine and thyroxine are mutually antagonistic in respect to tumour growth, the former being an important nutritional factor which is destroyed together with other tissue proteins in the augmented catabolism induced by the action of thyroxine.

The evidence obtained that the dose of arginine can be adjusted to balance the inhibitory effect of thyroxine is an indication that protein catabolism is

primarily involved, but another aspect which should be considered is the regulation of carbohydrate metabolism by the thyroid, hypo-activity having been shown to cause significant alteration [Wells, 1925], not only in sugar utilisation, but in all oxidative processes throughout the body. There is however no evidence that a reduction in blood-sugar diminishes the rate of tumour growth, indeed according to Bouin [1927] malignant tar tumours grew with great rapidity in rabbits receiving two rabbit units of insulin daily for six months, and the animals died 4 weeks after treatment was stopped. It may be contended that the greater malignancy of such tumours renders them useless for comparison with the tumour M63, but there is also experimental evidence that insulin has no inhibitory effect upon the growth of tumour grafts in mice; Cioffari and Piccaluga [1926] found that unless insulin was given in excessive doses (1/5 of a clinical unit daily) it had no effect; in some cases death occurred in 15 days and they concluded that hypoglycaemia alone does not materially affect the rate of tumour growth; the same workers report that a carbohydrate-rich diet is without effect in accelerating the rate of tumour growth. It is quite possible that although a reduction in blood-sugar is without effect a loss of balance between the catabolic and synthetic phase due to impaired oxidative processes would retard tumour growth [Warburg, 1926], but it is questionable whether it is actually anoxaemia which has been the cause of retarded growth in experiments on reduced oxygen tension in view of the compensatory mechanism which comes into play under such circumstances [Barcroft, 1925], although the extent of acclimatisation depends upon many factors and is not rapidly attained.

Before any general significance can be claimed for these results it is necessary to consider the reaction of the mouse towards thyroxine and the relation between this neoplasm (M63), and its host. Resistance of the mouse towards thyroxine has been recorded by Abderhalden and Wertheimer [1928], and it may be argued that the behaviour of a spontaneous neoplasm towards thyroxine would be in no way analogous to that of a tumour which was essentially a graft. If mice are relatively insusceptible to thyroxine this may explain the absence of any loss of weight during the experimental period, and indicate that the same degree of hyperthyroidism would not be tolerated by other mammals without the appearance of toxic symptoms. That thyroxine might cause resorption of a graft and therefore reduce the growth of this tumour appears a valid argument, but it must be remembered that treatment was never instituted until the graft was well established, 10 days after implantation, and that actual disappearance of the graft did not occur, although the treatment materially affected its subsequent rate of growth. Tumours which had already attained a large size, such as those previously treated with arginine (*e.g.* Exp. 4) continued to grow under treatment with thyroxine, a temporary cessation being followed by a sharp rise in the curve of growth. In view of the fact that treatment was limited to a period of 12 days the full results which might follow the reversal of treatment could not be attained,

since termination of the experiment is necessitated by ulceration of tumours in one of the more rapidly growing groups.

SUMMARY.

In a series of experiments using 165 mice it was found that injections of thyroxine retarded the rate of tumour growth as compared with untreated controls; this was not accompanied by any loss of body weight during a 12-day period of treatment. Substitution of arginine for thyroxine caused a renewal of rapid growth, but if arginine had been given previously the substitution of thyroxine did not cause a permanent deflection of the curve. If both were given to the same animal the rate of growth was similar to that of untreated controls if arginine was given daily, but if given less often the effect of thyroxine predominated, indicating an antagonism in the effect of these two substances in respect to tumour growth.

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